ZOOLOGICAL SCIENCE 6: 805–808 (1989)

[COMMUNICATION]

Immunohistochemical Studies of Juxtaglomerular Cells and the Corpuscles of Stannius in the Eel, *Anguilla japonica*

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ABSTRACT—The localization of immunoreactive renin in the juxtaglomerular cells (JGC) and the corpuscles of Stannius (CS) was investigated in the Japanese eel *Anguilla japonica*. Anti-serum against mouse submandibular gland renin raised in rabbits was used for the immunohistochemistry. Immunoreactive JGC were demonstrated in the wall of the glomerular afferent arterioles and the samll arterial branches of the kidney. However, renin-immunoreactivity could not be detected in CS.

INTRODUCTION

The juxtaglomerular apparatus is not so well developed in non-mammalian vertebrates as in the mammals [1]. In the teleostean kidney, juxtaglomerular cells (JGC) have been observed in the walls of the renal arterioles and/or small arteries; macula densa and the extraglomerular mesangium are not found [2]. Localization of renin in the granules of JGC was first demonstrated in mammals by fluorescent antibody technique [3]. Although the JGC are known as renin-containing cells in mice [4, 5], rats [6, 7], and humans [8, 9], little recent information is available concerning the existence of renin-containing cells in non-mammalian kidneys [10]. However, renin-immunoreactive cells were recently demonstrated in the carp [11].

The corpuscles of Stannius (CS) are unique endocrine glands of bony fishes. Since the removal of the CS was reported to induce hypercalcemia in the European eel [12], many investigations have indicated that the CS relate in calcium regulation in fishes [13]. The active principle of the CS has been termed hypocalcin [14] and several candidates for hypocalcin has been proposed: a small glycopeptide with a molecular weight of 3,000 [15], renin-like substance and/or angiotensin-like substance [16], acid stable protein with a molecular weight greater than 10,000 [17, 18], mammalian-PTH-like substance [19], and a glycoprotein with a molecular weight of 39,300 [20]. Histologically, granules in the CS cells are stained with Bowie's staining, which also stains granules in the JGC of the teleostean kidney [21, 22]. Renin-like activity was found in the CS of the carp and Japanese goosefish [23]. The amino acid sequences of angiotensin I (AI) obtained by incubating CS or kidney extracts with homologous plasma in chum salmon and the Japanese goosefise have identified [24, 25]. An angiotensin II-like substance, a member of the renin-angiotensin system, was found in the cells of CS of the rainbow trout immunohistochemically [26]. However, the presence of renin was not examined immunohistochemically in the CS.

In the present study, the presence of renin in the kidney and the CS of the Japanese eel, *Anguilla japonica* was studied by immunohistochemical methods.

Accepted September 24, 1988
Received June 7, 1988

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MATERIALS AND METHODS

Japanese eels, *Anguilla japonica* were used. Fish were anesthetized by MS 222. The kidney tissue associated with the CS was fixed in Bouin's fluid for 2 hr, embedded in paraffin, and sectioned at 4 μm. Rabbit anti-mouse submandibular gland renin antiserum was supplied through the courtesy of Prof. Kazuo Murakami, Institute of Applied Biochemistry, Tsukuba University [27, 28]. Its reliability was previously demonstrated in an immunohistochemical study [10, 11]. Immunohistochemical staining was carried out as previously described [29]. In brief, the sections were deparffinized and hydrated, and then the endogeneous peroxidase activity was blocked by exposure to methanol containing 0.2% H_2O_2 for 30 min. After being washed, the slides were incubated overnight with the antiserum diluted in phosphate-buffered saline to 1:500. The slides were then exposed to a biotinylated anti-rabbit immunoglobulin antiserum (dilution 1:500), avidin (dilution 1:1,000), and biotinylated horseradish peroxidase complex. The reagents were purchased from Vector Laboratories, Inc. (Vectastain, Burlingame, California). We developed the peroxidase reaction by incubating the slides in 0.5% H_2O_2 and 0.02% 3,3′-diaminobenzidine tetrahydrochloride for 10 min. Control sections were incubated with non-immune rabbit serum instead of anti-renin serum.

RESULTS AND DISCUSSION

The cells resembling JGC in the wall of the glomerular afferent arterioles and the small arterial brances in the eel kidney were intensely immunoreactive to anti-renin serum (Fig. 1). The immunoreactive JGC were located extensively and dispersedly in some portion in the afferent arterioles at the juxtaglomerular region and also in the small arterial branches locating apart from the vascular poles of glomeruli. They were detectable along the tunica media of the blood vessels (Fig. 2). The above features are consistent with those found in the previous investigations on the JGC of teleostean kidney [1].

A pair of eel CS were located on or in the ventral side of posterior kidney near its attachment

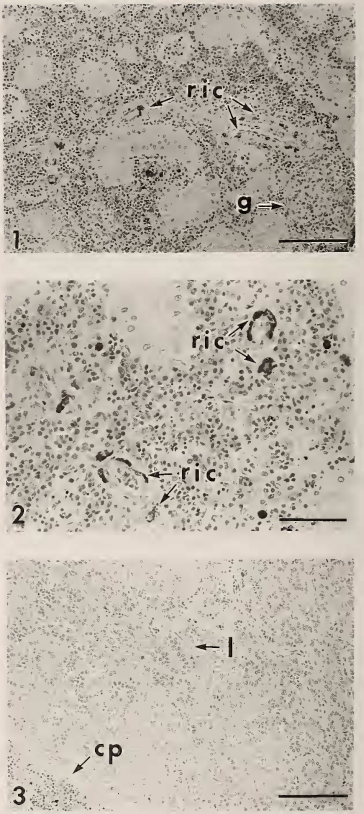

Fig. 1. Renin-immunoreactive cells (ric) resembling juxtaglomerular cells are observed in the wall of glomerular afferent arterioles and the small arterial branches of the kidney in the Japanese eel, *Anguilla japonica*. g: glomerulus. Scale, 100 μm.

Fig. 2. High magnification of some renin-immunoreactive cells (ric) in the wall of glomerular afferent arterioles of ell kidney. Scale, 50 μm.

Fig. 3. Corpuscles of Stannius of the Japanese eel. No renin-immunoreactive cells. l: lobule of CS. cp: capsule of the CS. Scale, 100 μm.

to the posterior cardinal vein. No renin immunoreactivity was detected in the eel CS even though immunoreactivity occurs in the kidney tissue in the same section (Fig. 3). This result suggests that the eel CS do not contain any substances which react with anti-mouse submandibular gland renin serum.

Renin-like activity has been found in the CS of the carp and Japanese goosefish [23]. Moreover, both a homogenate of carp CS and angiotensin-like substances which were formed by incubating the eel CS extract with homologous plasma produced hypocalcemia in Japanese eel [16]. Further, [Asp[1], Val[5], Asn[9]] AI and [Asn[1], Val[5], Asn[9]] AI were obtained chemically by incubating the CS or the kidney extracts with homologous plasma in the chum salmon [24] and [Asn[1], Val[5], His[9]] AI in Japanese goosefish [25]. However, renin-immunoreactivity of the CS could not be detected in the present study, even though the JGC of the kidney showed a positive reaction in the same section. It may be one of the possibilities that renin activity in the CS is too weak to be detected by the present immunohistochemical method. Moreover, the CS tissue, unlike the kidney, may require a different fixative for the best preservation of its cell inclusions. On the other hand, it was reported recently that the incubation of CS homogenate with homologous plasma did not result in any angiotensin-like peptides in the eel, *Anguilla australis* [30]. It is important to clarify whether renin is present in the CS or not. Therefore, further studies using different fixatives and antisera and required.

In the present investigation, it is evident that in the eel, the renin-immunoreactive cells resembling JGC are localized in the wall of the glomerular afferent arterioles and the small arterial branches of the kidney, but renin-immunoreactive cells are not detected in the CS.

ACKNOWLEDGMENTS

We are grateful for the generous gift of the anti-mouse submandibular gland renin serum by Prof. Kazuo Murakami, Institute of Applied Biochemistry, Tsukuba University, Japan. We wish also to express our gratitude to Prof. W. S. Hoar, University of British Columbia, for his critical reading of the manuscript.

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